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Antioxidant and Antiradical Properties of *Rhabdosciadium anatolyi* Flowers and Contents of Vitamin, Trace Element and Mineral

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ABSTRACT

The aim of this study was to investigate the antioxidant and antiradical activity of the *Rhabdosciadium anatolyi* (*R. anatolyi*) flowers, an endemic plant grown in the Eastern Anatolia Region of Turkey, by determining the level of vitamins E and C, mineral (Ca, Na, Mg, P, K), and trace elements (Mn, Zn, Cu, Fe, V, Cr, Mo, Co, Sr, Pb, Ti, Tl, Sn, Cd, As). Within the scope of the study, mineral and trace element analyzes were carried out by ICP-OES and AAS, Vitamin E by HPLC, Vitamin C, total phenolic content, total flavonoid, antioxidant capacity, hydrogen peroxide, DPPH, ABTS, superoxide, hydroxyl and hemolysis of erythrocytes with phenylhydrazine, radical scavenging activity of the *R. anatolyi* flowers methanol extract were determined spectrophotometrically. According to the results, α-tocopherol, ascorbic acid, phenolic content, flavonoid content and total antioxidant activity of the *R. anatolyi* flowers were 3,99 ± 0,35 µmol kg^{-1}, 346.27 ± 6.51 mg 100 g^{-1}, 21.94 ± 0.37 mg gallic acid g^{-1}, 5.60 ± 0.13 mg quercetin g^{-1} ve 29.65 ± 0.26 mM ascorbic acid g^{-1}, respectively. Consequently, the mineral, trace element, vitamin E and vitamin C, total phenol and flavonoid levels in the *R. anatolyi* flowers were high, and the *R. anatolyi* flowers methanol extract effectively inhibited free radicals. From this point of view, it is thought that it can be used in the preventive treatment of many diseases that may be caused by free radical species and that these data will be a reference for other studies.

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Keywords
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Rhabdosciadium anatolyi Çiçeğinin Antioksidan ve Antiradikal Özellikleri ile Vitamin, İz Element ve Mineral İçerikleri

ÖZET

Bu çalışmanın amacı, Türkiyenin Doğu Anadolu Bölgesi'nde yetişen, endemik bir bitki olan *Rhabdosciadium anatolyi (R. anatolyi)* çiçeğinin antioksidan, antiradikal aktivitesi-ile E ve C vitaminleri, mineral (Ca, Na, Mg, P, K) ve iz element (Mn, Zn, Cu, Fe, V, Cr, Mo, Co, Sr, Pb, Ti, Tl, Sn, Cd, As) düzeylerini belirlemektir. Çalışma kapsamında mineral ve iz element analizleri ICP-OES ve AAS ile E vitamini HPLC ile C vitamini, *R. anatolyi* çiçeği metanol ekstraktının toplam fenolik içeriği, toplam flavonoid içeriği ve antioksidan kapasitesi, hidrojen peroksit, DPPH, ABTS, süperoksit, hidroksil ve eritrositlerin fenilhidrazin ile hemoliz oluşumu sonucu *R. anatolyi* çiçeğinin radikal süpürücü aktivitesi spektrofotometrik olarak tespit edildi. *R. anatolyi* çiçeğinin α-tokoferol, askorbik asit, fenolik ve flavonoid içeriği ile toplam antioksidan aktivitesi sırasıyla 3,99 ± 0,35 µmol kg^{-1}, 346.27 ± 6.51 mg 100 g^{-1}, 21.94 ± 0.37 mg gallik asit g^{-1}, 5.60 ± 0.13 mg kuersetin g^{-1} ve 29.65 ± 0.26 mM askorbik asit g^{-1} olarak tespit edilmiştir. Sonuç olarak *R. anatolyi* çiçeğinin içeriğinde mineral, iz element, E ve C vitaminleri, toplam fenol ve flavonoid düzeylerinin yüksek olduğu, *R. anatolyi* çiçeği metanol ekstraktının serbest radikalleri inhibe etmede etkili olduğu

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Anahtar kelimeler
Antioksidan
İz element
Rhabdosciadium anatolyi
Serbest radikal
Vitamin

belirlendi. Bu açıdan bakıldığında serbest radikal türlerinin neden olabileceği birçok hastalığın koruyucu tedavisinde kullanılabileceği ve ayrıca bu verilerin başka çalışmalar için bir referans olacağı düşünülmektedir.

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INTRODUCTION

With the first civilizations, human beings have taken nature and events in nature under a keen observation. The first observation here is that it has been more or less determined that the assets in the environment would be used as raw and cookable, and that medicinal quality substances can be obtained. When we look at the historical process, the direct and indirect contribution of plants in meeting the basic needs of human beings such as nutrition, shelter, treatment and clothing is an important phenomenon (Küçüker, 2015). In general, it is a fact that plants are the first natural treatment material that people find healing when they get sick, in both in the past and today (Tanker et al., 1998; Mohammed et al.,2020).

Food products are in a critical position to strengthen immune system and live in a way that will increase health level to higher levels. In addition to nutrition, functional foods taken into the organism with nutrients have therapeutic and protective effects from diseases with their special active substance content. In recent years, it is seen that studies on traditional medicine have intensified and the active ingredients in these nutrients have positive effects on the immune system with their secondary compounds (Gürsel, 2014).

Although the treatment of living organisms with plants goes back to ancient times, herbal treatment has gained even more importance with the acceleration of studies on the damage caused by free radicals on living organisms. Because free radicals are a process in which both endogenous and exogenous factors that the living organism is exposed to, emerge at the end of a series of biochemical processes (Dröge, 2002). It is only possible for a living organism to turn this process in its favor with a correct mechanism. If we say that plants are in a critical position in this mechanism, we would not be wrong at all.

600 genera belonging to the Apiaceae family have been recorded so far (Çağın, 2005). The *R. anatolyi* species is a member of the Apiaceae family, belonging to the genus *Rhabdosciadium* Boiss. This plant is an endemic species that spreads only at 2400-2800 m in the Hakkari province region of Turkey. It is known that the Apiaceae family has been used as medicine and food for a long time, as well as having the largest, most cosmopolitan and economic importance (Burnie, 1996; Hançer et al., 2017).

The aim of this study is to investigate the content of *R. anatolyi* flowers, which an endemic plant is growing around Hakkari province in the Eastern Anatolia Region, which has a rich flora, by various methods. For this purpose, total phenol, flavonoid and antioxidant capacity, vitamin E and C content, trace element and mineral levels of flowers methanol extract of *R. anatolyi* flowers were determined. On the other hand, DPPH, ABTS, superoxide (O_2-), hydrogen peroxide (H_2O_2), hydroxyl (HO·) and anti-hemolytic activity methods were used to determine the radical scavenging effect of *R. anatolyi* flowers.

MATERIALS and METHODS

Plant Material and Extraction Processes

The *R. anatolyi* flowers used in the study was collected in Hakkari Province, Yüksekova town, Sat Mountains, Şitazin Gera Mezin (Sat glacial lake/ Big lake) region, at an altitude of 2356 meters and at 37º 22' 41" N - 44º 10' 08" D coordinates. Species identification of the plant was carried out by Research Assistant Mehmet FIRAT at Van Yüzüncü Yıl University. The witness plant sample is stored in the herbarium with the code 34041 (VANF). The plant specimen was dried in a cool shade, not exposed to sunlight. The dried part of the plant was pulverized in the herb grinder. 20 g were weighed on a precision balance and 400 mL of methanol (75%) was added. It was kept in a magnetic stirrer at 24 ºC (room temperature) for 48 hours. It was then filtered on filter paper. In order to remove the added methanol, it was treated in the evaporator and lyophilized at -65°C for 24 hours. The dried extract was stored at +4 ºC to be used in the study.

Determination of Vitamin C

The determination of the vitamin C amount of the *R. anatolyi* flowers was carried out by measuring spectrophotometrically at 521 wavelengths. Stock solutions of vitamin C were prepared in 4000 mg mL^{-1} metaphosphoric acid. After 1 gram of plant was weighed and transferred to the tubes, 2,4 dinitrophenylhydrazine was added and kept in a 90

°C water bath. After the water bath, sulfuric acid solution was added to them. The tubes were brought to room temperature and vortexed. Finally, measurements were made (Shimadzu UV 1800, Japan) and their absorbance was recorded. The absorbic acid concentrations of the samples were calculated using the obtained calibration chart (Brewster, 1984; Golubkina et al., 1989).

Determination of Vitamin E

Standart solutions and calibration

Stock solutions of vitamin E (α-tocopherol) were prepared in 500 µg mL^{-1}. Methanol was used to dilute the standard solutions. Calibration was calculated using linear regression analysis of the peak area of standard solution concentrations.

Extraction process

In the study, vitamin E amounts of *R. anatolyi* flowers were determined by modifying the method used, in accordance with (Sahin et al., 2005; Al-Saleh et al., 2006). From the dried plant samples and ground in the shade, 5 g was weighed and extracted with n-hexane and ethanol. 0.01% BHT was added to them, vortexed, and kept in the dark for 24 hours. Then centrifuged at +4 °C and 4000 rpm for 10 minutes. The supernatant was filtered using whatman filter paper. Then 500 µl of n-hexane was added and evaporated with nitrogen gas (37 °C). After drying, the residue was dissolved in a mixture of 0,2 mL methanol + tetrahydrofuran and made ready for analysis.

Chromatographic conditions

Analyzes, Gl Science C_{18} reverse phase HPLC column (250x4.6 mm ID), methanol + tetrahydrofuran (80:20) mobile phase, at a flow rate of 1500 µL min^{-1}, at a temperature of 25 °C. Thermo Scientific Finnigan Surveyor model in high performance liquid chromatography, using a PDA array detector, applications in a volume of 100 µL in dark-colored vials in tray autosampler (-8 °C) were performed at 290 nm α-tocopherol. Chromatographic analysis concentrated by isocratic elution (40 ºC).

Determination of Total Phenol Content

The Folin-Ciocelteu (FCR) marker was used to determine the total phenol content of the *R. anatolyi* flowers extract, in accordance with (Yi et al., 1997; Gamez-Meza et al., 1999). After adding 0.3 mL of 2% Na_2CO_3 to the flowers extract samples prepared by diluting with methanol, 0.1 mL of foline reagent was added and incubated for 2 hours at room temperature. The absorbances of the samples were read at 765 nm wavelength. Phenolic contents were expressed as gallic acid equivalents per weight (mg GAE g^{-1}) of the prepared extract.

Determination of Total Flavonoid Content

In order to determine the flavonoid content of the *R. anatolyi* flowers extract used in the study, 100 µL of potassium acetate was added to 0.5 mL of the stock solution diluted with methanol, and 0.1 mL of $Al(NO_3)_3$ and 4.6 mL of ethanol were added. At the end of this process, the solutions were vortexed and incubated for 40 minutes at room temperature. Finally, the absorbances of the samples were read against the control sample at a wavelength of 415 nm (Lamasion et al., 1990). The total favonoid amounts of the samples were given as mg g^{-1} as quercetin equivalent.

Determination of Total Antioxidant Capacity

The spectrophotometric method developed according to Prieto et al. (1999) was modified and used for the quantitative determination of the total antioxidant capacity of the *R. anatolyi* flowers extract. The main goal of this method is based on the reduction of acidic Molybdenum (VI) to Molybdenum (V) and the formation of a green colored phosphate/Molybdenum (V) composition at acidic pH. 0.2 mL of the flowers extract of the *R. anatolyi* flowers diluted with methanol in different concentrations was taken, and 2000 µL of reagent solution (0.6 M sulfuric acid, 0.028 M sodium phosphate and 0.004 M ammonium molybdate) was added to them, and then they were kept in a water bath at 95 ºC for 90 minutes. The samples were cooled at room temperature in an ice bath and read against the control sample at a wavelength of 695 nm. Antioxidant capacities of the samples were given as mM ascorbic acid g^{-1}.

DPPH Radical Scavenging Capacity

This method, which is used for the determination of antioxidant activity, is based on the principle of changing the purple color as a result of adding any synthetic antioxidant compound to the DPPH radical solution prepared with methanol (Cuendet et al., 1997; Chen et al., 2009). In case determine the DPPH radical scavenging feature of the *R. anatolyi* flowers extract, 5 mL of 0.004% DPPH solution was added to the solutions of different concentrations prepared by diluting with methanol and incubated for 30 minutes at room temperature. The absorbances of the samples were read against the control sample at a wavelength (517 nm). The % inhibition values obtained were plotted against the concentration and the values (IC_{50}) of *R. anatolyi* flowers extracts that inhibited DPPH radical by 50% were determined. Butylated hydroxy toluene (BHT) was used as a positive control.

$$\text{İnhibisyon (\%)} = \left[\frac{A_{blank} - A_{sample}}{A_{blank}}\right] \text{x } 100$$

ABTS Radical Scavenging Capacity

ABTS radical is based on the principle of decreasing color intensity by scavenging it by a compound obtained by the reaction of ABTS salt and $K_2S_2O_8$, a strong oxidizing agent (Re et al., 1999). The $ABTS^{\cdot+}$ radical scavenging effect of the *R. anatolyi* flowers extract was performed with the prepared 100 mM phosphate buffer with (pH: 7.4). 2.45 mM potassium persulfate solutions were prepared with 2 mM ABTS solution. Then, these two solutions were mixed and mixed with a magnetic stirrer for 10-18 hours at room temperature/dark environment. The prepared solution was calculated by reading its absorbance in the spectrophotometer at 734 nm. The stable free radical scavenging ability of the molecules was done with trolox, a synthetic antioxidant. For the control preparation, the ABTS solution was diluted with buffer (pH: 7.4) so that the absorbance was 0.70 at 734 nm. $ABTS^{\cdot+}$ radical scavenging activity (%) was calculated with the help of the following formula.

$$\text{İnhibisyon (\%)} = [\frac{A_{blank} - A_{sample}}{A_{blank}}] \text{x } 100$$

Determination of Hydrogen Peroxide (H_2O_2) Scavenging Activity

The hydrogen peroxide removal activity of the extract of the flowers part of the *R. anatolyi* flowers prepared with methanol was determined by reading it at a wavelength of 230 nm with a spectrophotometer device. First, 43 mM hydrogen peroxide (H_2O_2) solution was prepared in the prepared phosphate buffer (pH: 7.4). The volume of the *R. anatolyi* flowers extract taken at different concentrations and BHT solutions, which is the standard antioxidant substance used in the study, was completed with a buffer solution up to 0.4 mL. Then 600 µL of hydrogen peroxide solution was added. After 10 minutes of incubation at room temperature, the decreasing amount of hydrogen peroxide was recorded at 230 nm (Ruch, 1989).

Superoxide ($O_2^{\cdot}$) Radical Scavenging Capacity

The scavenging effect of the *R. anatolyi* flowers extract on superoxide anion radicals was determined by spectrophotometric measurement of nitro blue tetrazolium (NBT) at 560 nm (Zhishen et al., 1999). As a result of testing the solutions prepared at different concentrations, the most appropriate 45 µg mL^{-1} was prepared with phosphate buffer of 0.05 M pH: 7.8, so that the concentrations of the standard antioxidant substance BHT solutions to be compared with were the same. Equal proportions of riboflavin, L-methionine and NBT (200 µl) were added to these prepared solutions. The resulting reaction mixture was exposed to fluorescent light for 40 minutes at room temperature. The scavenging effect of the *R. anatolyi* flowers extract on superoxide anion radicals was compared with that of BHT, which has antioxidant properties. Absorbance was recorded at 560 nm against the water blank. The % inhibition value according to the change in the absorption of the control was determined according to the formula below.

$$\text{İnhibisyon (\%)} = [\frac{A_{blank} - A_{sample}}{A_{blank}}] \text{x } 100$$

Hydroxyl ($OH^{\cdot}$) Radical Scavenging Capacity

2.8 mM deoxyribose, 0.001 M $FeCl_3$, EDTA, ascorbic acid and H_2O_2 solutions were added to the flowers part samples of the *R. anatolyi* flowers prepared from different concentrations of the extract in equal proportions and finalized. Volume was made up with 1 mL of 20 mM phosphate buffer (pH: 7.4). After vortexing, the reaction mixture was incubated at 37 °C for 1 hour. Then, 1 mL of TBA and 1 mL of TCA were added and vortexed again, and then boiled at 100 °C for 35 minutes. The absorbance of the colored mixture formed by the released MDA with TBA was read against the control sample at 532 nm (Kunchandy and Rao., 1990). IC_{50} values were determined by plotting the % inhibition values obtained against different concentrations of the plant extract. The results were compared with BHT. The % inhibition value according to the change in the absorption of the control was determined according to the formula below.

$$\text{İnhibisyon (\%)} = [\frac{A_{blank} - A_{sample}}{A_{blank}}] \text{x } 100$$

Hemolysis of Erythrocytes by Phenylhydrazine and Radical Scavenging Activity of *R. anatolyi* Flowers

1 mL of phenylhydrazine, 0.1 mL of 20% PCV, 1,850 mL of buffer were added to the samples prepared from different concentrations of the methanol extract of the flowers part of the *R. anatolyi* flowers. After incubation at 37 °C for 1 hour, it was centrifuged at 4000 rpm for 10 minutes. After the supernatant was transferred to other tubes, its absorbance at 540 nm was read against the control sample. Results were compared with BHT (Valenzuela, 1977).

Trace Element and Mineral Determination

Quantification of the minerals in the flowers of the *R. anatolyi* flowers was carried out using the dry burning method. The flowers part of the plant, which was dried and ground before, was weighed according to this method and placed in porcelain crucibles. 2 mL of ethyl alcohol-sulfuric acid mixture was added to each sample. The prepared samples were burned in a fume hood. Then it was left in the ash furnace set at 250 °C. Porcelain crucibles placed in the muffle furnace were started at 250 °C and the temperature was increased by 50 °C every hour to 550 °C. While

the temperature was at 550 ºC, porcelain crucibles were kept open in the muffle furnace until the next day. Then, 5000 µL of hydrochloric acid (HCl) was added to them. Finally, it was filtered on filter paper and made ready for elemental analysis. Analyzes of Ca, K, Mg, Na and Fe elements were performed using Atomic Absorption Spectrometer (AAS). In addition, P, Zn, Cu, Co, Mn, Cd, Pb, Cr, Ti, Sr, As, Tl, Sn, Mo and V elements were analyzed using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).

Statistical Analyses

The means and standard error of the data were expressed as (X ± SEM). Group plots were created by finding mean and standard error values (X ± SEM). Nonlinear regression analysis was used to determine IC_{50} values. Measurements of the samples were performed in triplicate.

RESULTS and DISCUSSION

To determine the antioxidant properties of methanol flowers extract of *R. anatolyi* flowers, total antioxidant capacity, total phenolic and total flavonoid contents, DPPH, ABTS, hydroxyl radical, superoxide radical, hydrogen peroxide and anti-hemolytic activity were measured. In addition, *R. anatolyi*'s vitamin E and C, element (Fe, Zn, Cu, Co, Mn, Cd, Pb, Cr, Ti, Sr, As, Tl, Sn, Mo and V) and mineral (Ca, K, Mg, Na and P) levels were determined and the results are shown in Tables 1 and 2.

Table1. Vitamin E and C, total phenolic and flavonoid content of *R. anatolyi* flowers, total antioxidant capacity, element (Cu, Zn, Fe, Co, Mn, Cd, Pb, Cr, Ti, Sr, As, Tl, Sn, Mo and V) and mineral (Ca, K, Mg, Na and P) levels

Çizelge 1. R. anatolyi çiçeğinin E ve C vitamini, toplam fenolik ve flavonoid içeriği, toplam antioksidan kapasitesi, element (Cu, Zn, Fe, Co, Mn, Cd, Pb, Cr, Ti, Sr, As, Tl, Sn, Mo ve V) ve mineral (Ca, K, Mg, Na ve P) düzeyleri

Parameters	*Rhabdosciadium anatolyi* ($\bar{X}$ ± SEM)
α-tocopherol (µg g^{-1})	3.99 ± 0.35
Vitamin C (mg 100 g^{-1})	346.27 ± 6.51
Total phenolic content (mg GA g^{-1})	21.94 ± 0.37
Total flavonoid content (mg QE g^{-1})	5.60 ± 0.13
Total antioxidant capacity (mM A.A g^{-1})	29.65 ± 0.26
Sn (µmol kg^{-1})	0.22 ± 0.086
V (mmol kg^{-1})	0.017 ± 0.00033
Ti (mmol kg^{-1})	0.066 ± 0.0028
Cr (mmol kg^{-1})	0.012 ± 0.00026
Cu (mmol kg^{-1})	0.079 ± 0.0061
Sr (mmol kg^{-1})	0.088 ± 0.0059
As (µmol kg^{-1})	0.15 ± 0.062
Tl (µmol kg^{-1})	0.35 ± 0.063
Cd (µmol kg^{-1})	0.21 ± 0.044
Co (µmol kg^{-1})	2.56 ± 0.11
Pb (µmol kg^{-1})	1.81 ± 0.021
Mo (µmol kg^{-1})	0.83 ± 0.14
Mg (mmol kg^{-1})	3.64 ± 0.0063
Fe (mmol kg^{-1})	0.064 ± 0.0017
Mn (mmol kg^{-1})	0.43 ± 0.021
P (mmol kg^{-1})	0.081 ± 0.0028
Na (mmol kg^{-1})	2.03 ± 0.027
Ca (mmol kg^{-1})	2.61 ± 0.11
K (mmol kg^{-1})	2.09 ± 0.037
Zn (mmol kg^{-1})	0.38 ± 0.015

Values are expressed as mean ± standard error of mean (X ± SEM). Samples were performed in triplicate.

The graph showing the inhibition percentages and IC_{50} values of BHT, which is the positive control, of hydroxyl (OH^-) and hydrogen peroxide (H_2O_2) radicals of the methanol extract of *R. anatolyi* flowers is shown in Figure 2.

For *R. anatolyi* flowers and BHT, the values showing the hemolysis formation of erythrocytes with phenylhydrazine, the % inhibition of radical scavenging activity of *R. anatolyi* flowers and the change of IC_{50} values, and the percentages of superoxide radical inhibition corresponding to the concentration of the BHT standard (45 µg mL^{-1}) are given in Figure 3.

Figure 1 shows % inhibition and IC_{50} values of $ABTS^{\cdot+}$ radical and BHT and DPPH radical for *R. anatolyi* flowers and trolox.

Recent studies have focused on the term functional nutrients, and studies have been conducted and are still being carried out, showing that plants (phytochemicals) can contribute to health, especially the prevention of diseases such as cardiovascular, cancer and osteoporosis (Hasler, 2002; Çoşkun, 2011).

Table 2. % Inhibition and IC_{50} (µg mL^{-1}) values in the methanol extract of the flowers part of the *R. anatolyi* flowers compared with positive controls.

Çizelge 2. Pozitif kontrollerle karşılaştırıldığında R. anatolyi çiçek kısmına ait metanol ekstraktındaki % İnhibisyon ve IC_{50} (µg mL^{-1}) değerleri.

	Control	% Inhibition ($\bar{X}$ ± SEM)	IC_{50} (µgmL^{-1})($\bar{X}$ ± SEM)
DPPH·		71.27 ± 4.47	95.28 ± 1.61
	BHT	79.64 ± 3.49	71.18 ± 2.28
OH·		79.53 ± 0.17	136.96 ± 1.65
	BHT	79.30 ± 0.98	57.74 ± 8.22
H_2O_2		54.55 ± 0.61	29.81 ± 0.54
	BHT	55.16 ± 0.26	10.85 ± 2.28
ABTS		91.14 ± 0.20	29.44 ± 0.49
	Trolox	90.20 ± 0.34	51.75 ± 3.36
$PhNHNH_2$		53.19 ± 1.03	77.68 ± 2.23
	BHT	52.68 ± 0.17	63.03 ± 6.02

Values are expressed as mean ± standard error of mean (X ± SEM). Samples were performed in triplicate.DPPH: 2,2-difenil-1-pikrilhidrazil, OH: Hidroksil, H_2O_2: Hidrojen peroksit, ABTS: 2,2'-azinobis (3-etilbenzotiazolin-6-sülfanot), $PhNHNH_2$: Fenilhidrazin.

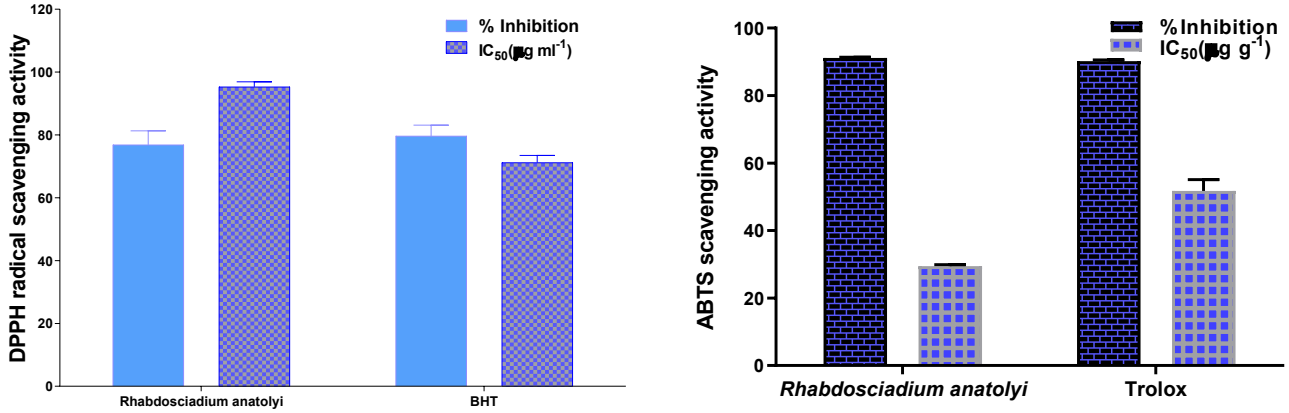

Figure 1. % inhibition of DPPH and ABTS radicals and IC50 values for BHT and trolax of *R. anatolyi* flowers

Şekil 1. R. anatolyi çiçeğinin BHT ve trolaks için DPPH ve ABTS radikallerinin % inhibisyonu ve IC_{50} değerleri.

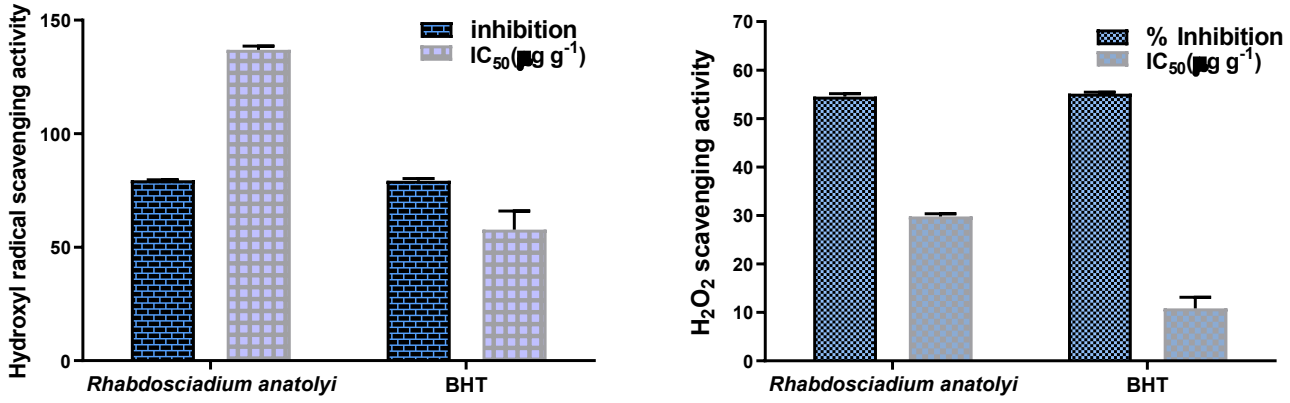

Figure 2. Graph showing the change in hydroxyl radical scavenging activity of *R. anatolyi* flowers and BHT, and % inhibition and IC_{50} values of BHT and hydrogen peroxide radical scavenging activity.

Şekil 2. R. anatolyi çiçeğinin ve BHT'nin hidroksil radikali süpürme aktivitesi ile BHT ve hidrojen peroksit radikali süpürme aktivitesine ait % inhibisyon ve IC_{50} değerlerindeki değişimi gösteren grafik.

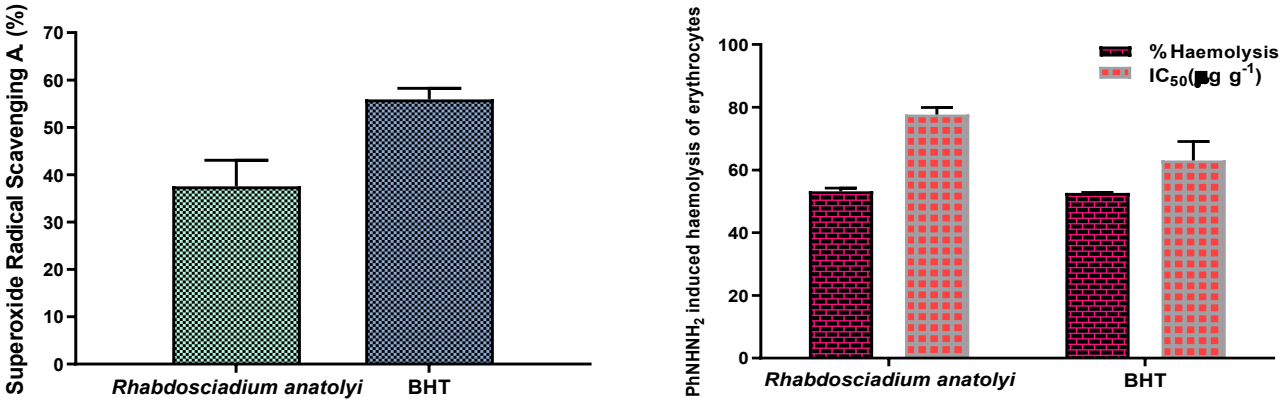

Figure 3. Graph of % inhibition and IC_{50} values showing % inhibition change of *R. anatolyi* flowers superoxide radical and hemolysis of erythrocytes with phenylhydrazine for R. anatolyi plant and BHT.

Şekil 3. R. anatolyi çiçeğinin süperoksit radikalinin % inhibisyon değişimi ve R. anatolyi çiçeği ile BHT için eritrositlerin fenilhidrazin ile hemoliz oluşumu gösteren % inhibisyon ve IC_{50} değerlerine ait grafik.

Carotenoids, flavonoids and polyphenols, which are phytochemicals or phytonutrients, not only protect plants from many diseases and fungi, but also add features such as color, smell and taste to plants (Mosley, 2018). These phytochemical compounds found in plants prevent the oxidation of free radicals, which are produced by lipids, carbohydrates and proteins as waste products as a result of various reactions in the organism (Çoban & Patır, 2010). Phenolic compounds are powerful and natural antioxidants that scavenge these free radicals formed in various conditions. In this study, total phenol, total flavonoid and total antioxidant capacities of *R. anatolyi* flowers were determined. The total amount of phenol was determined using the gallic acid standard graph, the total flavonoid quercetin standard graph, and the total antioxidant was determined using the ascorbic acid standard graph. Total phenol, flavonoid and antioxidant amounts were determined as 21.94 ± 0.37 mg gallic acid g^{-1}, 5.60 ± 0.13 mg quercetin g^{-1} and 29.65 ± 0.26 mM ascorbic acid g^{-1}, respectively.

Apart from proteins, lipids and carbohydrates, which are macronutrients, vitamins are compounds that are essential to perform some special cellular events and are needed at trace levels in the organism (Ferrier, 2019). Vitamin C, which is included in the water-soluble class of vitamins, is important in two respects; The first is that it is a very powerful antioxidant and the other is that it is essential for some vertebrates, including humans (Korkmaz et al., 2012). Apart from the known role of vitamin C in collagen synthesis in connective tissues, studies on its interactions with various chemicals and metal ions have shown that free radicals have important roles in reactions involving electron transport and membrane biochemical strengthening (Hacışevki, 2009). The primary function of vitamin E is to play an antioxidant role in maintaining the non-enzymatic oxidation of cell components. In addition, studies have shown that ascorbic acid regenerates active vitamin E (Ferrier, 2019). The amount of vitamin C in the flowers extract of the *R. anatolyi* flowers was determined as 346.27 ± 6.51 mg 100 g^{-1}, and the value of vitamin E was determined as 3.99 ± 0.35 µg g^{-1}. In the literature review, there is a not study on vitamins E and C related to both *R. anatolyi* flowers and closely related species *R. microcalycinum*, *R. oligocarpum*, *R. urusakii*, *R. alignense*, *R. petiolare*, *R. aucheri*, *R. hizanense* (Fırat & Güzel, 2019) and *R. strauss* species. However, the vitamin C content of the closely related species *Chaerophyllum macropodum* was determined as 20.53 ± 5.08 mg 100 g^{-1} , in accordance with Tunçtürk et al., (2008). As a result, it was determined that the vitamin C content in the *R. anatolyi* flowers was more significant and at a good level compared to the other plant in question. In addition, the value of vitamin E will be a reference for the studies to be done in this plant species.

The living organism generally consists of elements that it needs both roughly in abundance and at trace levels. Carbon, hydrogen, oxygen and nitrogen are the most abundant elements. They constitute 96% of body weight (Wada, 2004; Keha & Küfrevioğlu, 2012). The essential trace elements of the human body include elements such as Zn, Cu, Se, Cr, Co, I, Mn and Mo. Although these elements make up only 0.02% of the total body weight, they play important roles, for example, as active enzyme centers or trace bioactive substances (Wada, 2004). Mg, Fe, Zn, Cu and Cr elements have biochemical functions that have the potential to affect the physical performance of living things. These elements function as structural or catalytic components of enzymes, regulating cellular energy and gas transport, antioxidant defense, membrane receptor functions, second messenger and integration of physiological systems. Thus, mineral elements regulate the use of macronutrients

(Lukaski, 2004). Trace elements, which have an important and critical position in the nutrition of living organisms, have a very complex historical process (Shkolnik, 2012). Because although there are elements whose usefulness to the organism has been defined, it is a fact that there are elements whose functions have not yet been fully explained. Some trace elements such as copper, zinc and selenium cover both humoral and cellular immunity (Berger et al., 1998). These essential elements interact directly with free radical formation and free radical scavengers (Bendich, 1993). In addition, antioxidant enzymes need trace elements such as Cu, Fe, Zn, Se to provide catalytic activity. It is known that glutathione peroxidase enzyme needs Se element, superoxide dismutase enzyme needs Fe, Cu and Zn elements and catalase enzyme needs Fe element.

In the study, the mineral and trace element levels of the *R. anatolyi* flowers were examined. Values found are Ca 2.61 ± 0.11 mmol kg^{-1}, K 2.09 ± 0.037 mmol kg^{-1}, Mg 3.64 ± 0.0063 mmol kg^{-1}, Na 2.03 ± 0.027 mmol kg^{-1}, Fe 0.064 ± 0.0017 mmol kg^{-1}, Mn 0.43 ± 0.021 mmol kg^{-1}, P 0.081 ± 0.0028 mmol kg^{-1}, Zn 0.38 ± 0.015 mmol kg^{-1}, V 0.017 ± 0.00033 mmol kg^{-1}, Ti 0.066 ± 0.0028 mmol kg^{-1}, Cr 0.012 ± 0.00026 mmol kg^{-1}, Cu 0.079 ± 0.0061 mmol kg^{-1}, Sr 0.088 ± 0.0059 mmol kg^{-1}, As 0.15 ± 0.062 µmol kg^{-1}, Tl 0.35 ± 0.063 µmol kg^{-1}, Cd 0.21 ± 0.044 µmol kg^{-1} Co 2.56 ± 0.11 µmol kg^{-1}, Pb 1.81 ± 0.021 µmol kg^{-1}, Mo 0.83 ± 0.14 µmol kg^{-1} and Sn 0.22 ± 0.086 µmol kg^{-1} contents. When these values were compared, it was determined that Mg > Ca > K > Na > Mn > Zn > Sr > P > Cu > Ti > Fe > V > Cr > Co > Pb > Mo > Tl > Sn > Cd > As. As a result, it was determined that the plant flowers contains both macrominerals and trace elements. When the mineral levels of the *R. anatolyi* flowers are compared with the similar species *Echinophora tenuifolia* L., it has been found that it has an important mineral such as Na element (<1 mg $gün^{-1}$). In addition, the presence of trace elements such as Co, Mo, which are needed at ultra-trace levels in living organisms, have been determined.

DPPH is a stable radical. The change in absorbance of the DPPH radical was measured and the absorbance corresponding to the concentration was plotted and determined from the non-linear curve. The sample amount that halves the DPPH concentration is determined in µgmL^{-1} and expressed as the IC_{50} value. IC_{50} antioxidant activity is defined as the effective concentration, which expresses the amount of antioxidant consumed to reduce the initial DPPH concentration by 50%. The lower the IC_{50} value, the higher the radical scavenging activity (Brand-Williams, W., 1995). The capacity of the methanol extract of *R. anatolyi* flowers, which we used within the scope of the study, to scavenge DPPH radical, which is a free radical that can react with compounds that can donate hydrogen atom, was investigated. The highest inhibition percentage was 71.27 ± 4.47 µg mL^{-1} for the plant, and 79.64 ± 3.49 µg mL^{-1} for BHT, the positive control. The IC_{50} value was 95.28 ± 1.61 µg mL^{-1} for the plant, and 71.18 ± 2.28 µg mL^{-1} for BHT, which was the positive control. When these IC_{50} values were compared, it was determined that it was effective close to the standard and the concentration value of the plant extract, which inhibited the DPPH radical by 50%, was low. Compared with other plant species, it has been determined that it has a good scavenging activity and has high and significant values in terms of DPPH radical scavenging activity.

Hydrogen peroxide (H_2O_2) is an oxidizing agent, non-reactive, and its main importance is that it is a source of hydroxyl radicals in the presence of reactive transition metal ions (Kumar, 2011). Hydrogen peroxide can easily pass through cell membranes and directly attack the cellular target. For; example high levels of hydrogen peroxide can inactivate the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase in mammalian cells. (Halliwell, 1992). The main damage that hydrogen peroxide can cause in the cell results in DNA fragmentation, single strand breakage and formation of DNA protein crosslinking. The hydrogen peroxide (H_2O_2) radical scavenging capacity of *R. anatolyi* flowers was investigated. The highest inhibition percentage of plant flowers methanol extract was determined as 54.55 ± 0.61 µg mL^{-1}, and 55.16 ± 0.26 µg mL^{-1} for BHT. Concentration values inhibiting half of the hydrogen peroxide (H_2O_2) radical concentration were determined as 29.81 ± 0.54 µg mL^{-1} for the plant and 10.85 ± 2.28 µg mL^{-1} for BHT. Peroxisomes, which are in an important position in neutralizing free oxygen radicals, are important in that they contain enzymes that synthesize hydrogen peroxide as well as enzymes that break down (Çoşkun., 2005). From this point of view, it is seen that the scavenging activity of hydrogen peroxide (H_2O_2) radical, which is a free radical, of *R. anatolyi* flowers methanol extract has a lower % inhibition compared to synthetically used BHT, but a higher IC_{50} value.

The superoxide (O_2-) anion radical is the radical that has been shown to form first in living things (Kılınç & Kılınç, 2002). This radical is much less reactive than the hydroxyl radical but can attack a number of biological targets. Therefore, this radical reacts with nitric oxide, a free radical produced by various cell types, particularly phagocytes and vascular endothelial cells, to give peroxynitric (ONOO-) (Halliwell, 1992). Methanol extract of *R. anatolyi* flowers was compared with BHT, a synthetic antioxidant with known antioxidant properties. % inhibition activity of superoxide anion (O_2.-) radical at a concentration of 45 µg mL^{-1} is as *R. anatolyi* > BHT and values were determined as 37.55 ± 5.49 µg mL^{-1}

for the plant and 55.91 ± 2.32 µg mL^{-1} for BHT. When the superoxide anion radical values were examined, it was determined that the methanol extract of the *R. anatolyi* flowers effectively removed the superoxide radical at the concentration in question, compared to the BHT of the same value.

The hydroxyl radical (OH-), which has a very short half-life, is perhaps the most reactive type of ROS identified in biological systems (Kılınç & Kılınç, 2002; Özcan et al., 2015). Where the hydroxyl radical is formed, it causes great damage to many molecules such as thiols and fatty acids, causing the formation of other different radicals. Namely, thiols in the natural antioxidant group, which are the subject of important researches such as anti-cancer, are oxidized in the presence of oxygen and sulfide radicals are released as the final product, as well as reagents such as superoxide radical and hydroxide radical (Kavas, 1994).

The hydroxyl (OH^-) radical scavenging capacity of *R. anatolyi* flowers was investigated. The highest inhibition percentage of the plant flowers methanol extract was determined as 79.53 ± 0.17 µg mL^{-1}, and 79.30 ± 0.98 µg mL^{-1} for BHT. Concentration values inhibiting half of the hydroxyl (OH^-) radical concentration were determined as 136.96 ± 1.65 µg mL^{-1} for the plant and 57.74 ± 8.22 µg mL^{-1} for BHT. It is seen that the scavenging activity of the hydroxyl (OH^-) radical, which is a strong free radical, of the plant methanol extract is slightly higher than the synthetically used BHT, but the IC_{50} value is almost half-lower. As a result, when the IC_{50} values were examined, it was determined that *R. anatolyi* > BHT and the hydroxyl (OH^-) radical scavenging activity of the plant did not have a good scavenging activity compared to BHT, which is a synthetic antioxidant.

ABTS exists in a non-radical and colorless form. However, by oxidation of persulfate (potassium or sodium persulfate) it forms a blue/green $ABTS^{\cdot+}$ radical. This radical is very stable. The generation of $ABTS^{\cdot+}$ radical cation forms the basis of one of the spectrophotometric methods applied to measure the total antioxidant activities of pure solutions (Re et al., 1999). The addition of antioxidants to the preformed radical cation reduces the antioxidant concentration of the $ABTS^{\cdot+}$ radical to some extent and on a time scale, and the duration of the reaction, depending on the antioxidant activity. Thus, the degree of color change as percent inhibition of the $ABTS^{\cdot+}$ radical cation is determined as a function of concentration and time and is calculated based on the reactivity of a synthetic antioxidant to be used as a standard under the same conditions (Re et al., 1999). The lightening of the blue/green color of the $ABTS^{\cdot+}$ cation radical occurs in the presence of antioxidants. $ABTS^{\cdot+}$ radical scavenging activity of trolox antioxidant, which we used as a comparison of *R. anatolyi* flowers methanol extract at the same rate, was investigated. The highest inhibition percentage of plant flowers methanol extract was determined as 91.14 ± 0.20 µg mL^{-1}, and 90.20 ± 0.34 µg mL^{-1} for trolox. The concentration values inhibiting half of $ABTS^{\cdot+}$ cation radical concentration were determined as 29.44 ± 0.49 µg mL^{-1} for the plant and 51.75 ± 3.36 µg mL^{-1} for trolox. These values show that plant methanol extraction has been found to scavenge $ABTS^{\cdot+}$ radical scavenging activity at a better level compared to the comparator trolox.

Phenylhydrazine is a hemolytic agent. Yellow to pale brown crystals or a yellowish oily liquid, phenylhydrazine is sparingly soluble in water and miscible with other organic solvents. Limited data on toxicokinetics indicate that phenylhydrazine is well absorbed by inhalation, oral and dermal routes and readily binds to hemoglobin in red blood cells. Therefore, exposure to phenylhydrazine can damage red blood cells, potentially causing anemia and damage to other tissues such as the spleen and liver (Anonymous, 2000). It was investigated whether R. anatolyi plant flowers has phenylhydrazine radical scavenging activity in terms of antioxidant as a result of hemolysis of erythrocytes with phenylhydrazine. As a comparison, the phenylhydrazine radical scavenging activity of BHT, a synthetic antioxidant, was investigated. Hemolysis formation of erythrocytes with phenylhydrazine of plant flowers methanol extract was determined as 53.19 ± 1.03 µg mL^{-1} for the plant, and 52.68 ± 0.17 µg mL^{-1} for BHT. The IC_{50} concentration values inhibiting half of the phenylhydrazine radical concentration were determined as 77.68 ± 2.23 µg mL^{-1} for the plant and 63.03 ± 6.02 µg mL^{-1} for BHT. These results show that both the % inhibition and IC_{50} value of the plant were higher than BHT and the radical scavenging capacity of the plant flowers methanol extract was found to be lower than BHT, a monohydric phenolic antioxidant.

CONCLUSION

When looking at the general study on *R. anatolyi* flowers, total phenolic, flavonoid and total antioxidant capacity, vitamin E and C content, trace element and mineral levels, and antioxidant scavenging status against some radicals were evaluated. The level of active content of each plant can certainly differ depending on the conditions such as soil structure, geographical location, climate in which it grows, apart from the family it is in. It has been determined that the methanol extract of *R. anatolyi* flowers is rich in vitamin C content, phenolic, flavonoid and total antioxidant capacity is at sufficient level, and trace element and mineral levels have a significant ratio. It is seen that the superoxide radical and $ABTS^{\cdot+}$ radical outperform the synthetic antioxidants used in comparison. On the other hand, when the DPPH,

hydrogen peroxide, hydroxyl and phenylhydrazine radical scavenging activities were compared with the synthetic antioxidants used for comparison, it was determined that the methanol extract of the plant flowers had antiradical activity when evaluated as a whole.

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Author's Contributions

Author 1: Plant collection, data interpretation, evaluation of analysis results, article writing, review and editing. Author 2: Methodology, validation, statistics, data improvement, and auditing. Author 3: Collecting plant material. All authors accept responsibility for all content.

Conflict of Interest Statement

There was no conflict of interest among the authors in this study.

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